Isolation of High Seed Inorganic P, Low-Phytate Soybean Mutants

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ABSTRACT

Phosphorous in soybean [*Glycine max* (L.) Merr.] seed is stored primarily as phytic acid, which is nutritionally unavailable to nonruminant livestock. The objective of this study was to isolate mutations that reduce soybean seed phytic acid P and increase seed inorganic P. Following treatment with ethyl methanesulfonate, M2 through M6 plants were screened for high seed inorganic P. Seeds of M2 plants high in inorganic P produced progenies high in inorganic P through the M6 generation. M6 progenies of one plant averaged 6.84 g kg^{-1} seed phytic acid and inorganic P varied from 2.34 to 4.41 g kg^{-1} or 60 to 66% of phytic acid P plus inorganic P. M6 progenies of a second plant averaged 10.89 g kg^{-1} phytic acid and varied from 1.21 to 3.84 g kg^{-1} inorganic P, representing from 47 to 51% of the sum of phytic acid P plus inorganic P. In contrast, nonmutant seeds of the check cultivar Athow contained 15.33 g kg^{-1} phytic acid and averaged 0.74 g kg^{-1} inorganic P, representing 15% of the sum of phytic acid P plus inorganic P. Low phytic acid and high inorganic P in these progenies should increase the nutritional value of soy meal and reduce excess P in livestock manure.

Soybean meal is an important source of protein in livestock feeds and is important in human nutrition as well. Most of the P in soybean seed is stored in the form of phytic acid (*myo*-inositol 1,2,3,4,5,6-hexaphosphate), and phytic acid P is nutritionally unavailable to nonruminant livestock (Erdman, 1979). Additionally, phytic acid is a strong chelating agent that can bind metal ions, reducing availability of iron, zinc, calcium, and magnesium (Brown and Solomons, 1991; Erdman, 1981; McCance and Widdowson, 1935; Mendoza et al., 1998). Soy meal is commonly supplemented with inorganic P, and more recently with the enzyme phytase to increase the bioavailability of P and of these metal ions (Adeola, 1995; Adeola et al., 1995).

Mutants of corn (*Zea mays* L.) and of barley (*Hordeum vulgare* L.) have been isolated with genetically reduced amounts of phytic acid in the seed (Ertl et al., 1998; Larson et al., 1998; Raboy and Gerbasi, 1996; Rasmussen and Hatzack, 1998). These *low phytic acid* (*lpa*) mutants have as much as a 70% reduction in seed phytic acid with corresponding increases in inorganic P. Substitution in feeds of wild-type or normal phytic acid grains with low-phytate grains demonstrates that grain phytic acid P is "nonavailable" P in nonruminant diets. Observed reductions in animal waste P were proportional to the genetically conferred reductions in grain phytic acid P. A growing number of poultry, swine, and fish nutrition studies have shown that the amount of "available P" in low-phytate grains, P absorbed and utilized by an animal, is increased in proportion to the decrease in grain phytic acid P. Depending on the diet formulation, animals can absorb from 25 to 50% more P from the low-phytate grain, and excrete that much less grain P as fecal P, when compared with the amount of P these animals absorb from normal grains (Ertl et al., 1998; Huff, et al., 1998; Pierce et al., 1998; Sugiura et al., 1999; Veum et al., 1998). Low-phytate soy feed formulations had a beneficial effect on zinc and copper absoption and status in infant rhesus monkeys (*Macaca mulatta*) (Lonnerdal et al., 1999). Iron absorption from tortillas made from genetically modified low phytate maize was greater than from wild-type maize in human diets (Mendoza et al., 1998). Supplementing swine diets with phytase also has resulted in a reduction of fecal P excretion (Lei et al., 1993).

Since corn-soybean meal and soy meal are commonly fed livestock rations, reducing phytic acid in soybean would contribute to more efficient livestock feeds and reduced P in livestock manure. Previous evaluations of soybean have not identified genotypes with low levels of phytic acid in the seed. A large number of previous studies have surveyed the total P and phytic acid P content of soybean cultivars and lines and those of other legumes (Raboy, 1997). These studies found that one can observe heritable variation for seed phytic acid P among soybean lines ranging up to 50% of the mean value. However, this variation mostly reflects variation in seed total P, with the proportion of seed total P found as phytic acid P remaining relatively constant. Raboy et al. (1984) reported that phytic acid P varied from 67 to 77% of total P among seeds of 38 soybean cultivars. Differences among cultivars in the percent of seed total P represented by phytic acid P were not statistically significant. Little variation in seed nonphytic acid P, equivalent to "available P" in nonruminant nutrition, has been observed in these surveys.

The objective of this research was to isolate soybean mutants with decreased phytic acid P and increased seed inorganic P, similar to the *low phytic acid* mutants isolated previously in cereal grains.

MATERIALS AND METHODS

Plant Culture and Screening for High Inorganic P (HIP) Seed

About 2500 seeds of the soybean breeding line CX1515-4 were soaked for 24 h in a 18 m*M* solution of ethyl methanesulfonate (EMS), a mutagenic agent. The breeding line CX1515-4 is an F_4 plant selection from the cross CRS3-998-24-1 × C1813. CRS3-998-24-1 is a selection from cycle 1 of a recurrent selection program for high seed protein (478 g kg^{-1} protein, dry basis). C1813 is a selection from the cross C1655 × 'Pella 86'. The parentage of C1665 is 'Nebsoy' (Williams et al., 1980) ×

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A75-305022, and A75-305022 is a selection from the cross 'Wye' × ['Amsoy' (Weber, 1966) × 'Wayne' (Bernard, 1966)]. Following the 24-h soak, seeds were rinsed in distilled water and planted in rows spaced 10 cm apart in a sand bench. Emerged seedlings were transplanted to clay pots containing a sand-soil-peat mixture, and these M1 plants were grown to maturity.

Seeds were harvested from about 1000 individual M1 plants and the M2 generation grown at the Purdue University Agronomy Research Center in 1996. Ten to 20 seeds from each M1 plant were sown in a Chalmers soil (fine-silty, mixed, mesic Typic Haplaquoll) in rows 1 m in length, spaced 0.75 m apart. At maturity, M3 seed were threshed from the pods of five M2 plants from each row.

M3 seeds from a total of 3,994 M2 plants were individually tested for the High Inorganic P (HIP) phenotype associated with homozygosity for a *low phytic acid* mutation. A preliminary analysis of seed produced by nonmutant soybean cultivars grown under standard field conditions indicated that these seed typically contained about 0.2 g inorganic P kg^{-1}. For purposes of comparison, each P fraction (inorganic P or phytic acid P) is expressed as its P content, atomic weight 31. These seed typically contain about 5 g or more total P kg^{-1}, so that inorganic P typically represents about 5% of total seed P. These data are similar to data reported in an earlier study (Raboy and Dickinson, 1987). Seeds were screened for those containing substantial increases in inorganic P as compared with the typically low levels found in nonmutant seeds.

For routine screening, one M3 seed representing each M2 was individually crushed, and extracted overnight in 2.5 mL 12.5% (v/w) TCA:25 m*M* $MgCl_2$ at 4°C, with gentle shaking. Extracts were allowed to settle for 30 min, and aliquots of each single-seed extract were assayed for inorganic P by a modification of the method of Chen et al. (1956), designed for use in microtitre plates. The assay total volume was 200 μL. A 10-μL aliquot of each single-seed extract was placed in a microtitre plate well, to which was added 90 μL DD H_2O, a 100 μL of colorimetric reagent that was 1 vol 3 *M* H_2SO_4, 1 vol 0.02 *M* ammonium molybdate, 1 vol 10% (v/v) ascorbic acid and 2 vol DD H_2O. Assays were incubated at room temperature for 1.5 h, and the results were scored visually for the presence or absence of HIP.

Each microtitre plate included five P standards made by appropriate dilutions of 1m*M* K_2HPO_4 to achieve (i) 0.0 μg P; 2) 0.15 μg P; (ii) 0.46 μg P; (iii) 0.93 μg P; and (iv) 1.39 μg P. In practice, nonmutant seeds give a visual result equal to or less than the second colorimetric standard (0.15 μg P). Any seed extracts testing higher than the third standard (0.46 μg P) were deemed HIP. Previous screening for low phytic acid mutants in cereal crops used 0.4 *M* HCl for seed extraction, but preliminary development of methods for use in soybean screening found that this reagent resulted in a low frequency of artificial results falsely indicating HIP in seed actually containing low levels of inorganic P. Few such artifacts were observed when using the TCA solution.

Initial Test for Correspondence of High Inorganic P and Reduced Phytic Acid

To provide an initial test of the correspondence between increased inorganic P and reduced phytic acid P, additional seeds from selected progenies were subjected to a more rigorous single-seed extraction and assay of inorganic P and phytic acid P. Single seeds were weighed, crushed and extracted overnight in 20 (v/w) 12.5% TCA:25 m*M* MgCl2 at 4°C, with magnetic stirring. Extracts were then sonicated for 1 min at 4°C. Following centrifugation (10 000 g, 10 min), inorganic P in the supernatants was assayed colorimetrically in triplicate as described by Chen et al. (1956).

Anion-exchange HPLC analyses of phytic acid in these single-seed supernatants was then performed using the following modification of the methods as described (Phillippy and Bland, 1988; Rounds and Nielsen, 1993). The supernatants were first diluted from 3- to 6-fold with DD H_2O (as needed to yield phytic acid peaks within the limits of the standard curve described below), filtered through Whatman No. 1 filter paper, and passed through Millipore HV 0.45-μm filters. Aliquots were then fractionated on a Dionex IonPac AS7 anion-exchange column, equipped with a Dionex IonPac AG7 guard column (Dionex Corp., Sunnyvale, CA), which had been equilibrated with 10 m*M* methyl piperazine, pH 4.0 (Buffer A). Phytic acid and other inositol phosphates were then eluted with the following gradient system at a flow rate of 0.5 min^{-1}:0 to 1 min 100% Buffer A; 1 to 26 min a concave gradient from 0 to 15% *M* $NaNO_3$, pH 4.0 (Buffer B); 21 to 41 min a linear gradient from 15 to 100% Buffer B. The column elutant was mixed with colorimetric reagent [0.015% (w/v) $FeCl_3$:0.15% (w/v) sulfosalicylic acid] at a flow rate of 0.5 mL min^{-1}, using an Alltech PEEK tee (Alltech, Inc., Nicholasville, KY) and a pulseless pump, and the mixture passed through a 290-cm reaction coil prior to peak detection via absorbance at 550 nm. Phytic acid peaks were identified as those eluting at the same time as standard phytic acid, and quantitated by the following standard curve, obtained via the analysis of four potassium phytate standards containing 25, 50, 75, and 100 n*M* potassium phytate; n*M* phytic acid $=1.30 \times 10^{-}5$ (Peak Area)+5.79, $R^2 = 0.997$.

Segregation of the HIP/low-phytate trait in M3 through M6 Progenies of M153 and M766

Two progenies were identified as containing putative *low phytic acid* mutations, M153 and M766. To test for segregation of the HIP trait in their decedents, the following variation of the above colorimetric assay for inorganic P was used. A 50-μL aliquot of a TCA extract was transferred to a test tube with a disposable cuvette, to which was added 950 μL distilled water plus 1 mL of colorimetric reagent as described above. After 1 h, intensity of blue color was observed and visually compared with the pale colored solution from seed of 'Athow' (Wilcox and Abney, 1997) that has normal levels of inorganic P. In subsequent tests, color was measured after 1 h at 820 nm wavelength in a spectrophotometer. In the M6 generation, inorganic P was determined on each of four to six seeds from each M6 plant. In all tests, comparisons were made with inorganic P levels in the cultivar Athow. Seeds with >1.20 g kg^{-1} P were classified as high, and those with <0.90 g kg^{-1} P were classified as low in inorganic P.

Remnant seed of M3 progenies that tested for the HIP phenotype were grown in the greenhouse at West Lafayette, IN, in 1996 to 1997, with one seedling per 15-cm clay pot. Seeds from individual M3 plants, either high or low in inorganic P, were seeded in progeny rows at the Agronomy Research Center, West Lafayette, IN, to produce the M4 generation. Rows were spaced 1 m apart, and length of the row was determined by the number of M4 seeds harvested from each M3 plant. The M5 generation was grown in the greenhouse during the 1997 to 1998 winter months, and the M6 generation grown in the field at West Lafayette, IN, in 1998, using plots similar to those in 1997. The number of plants per M5 progeny row varied from 5 to 30.

Individual plants were harvested from these rows at maturity. A composite of four to six seeds from specific M6 plants were analyzed for inorganic P with methods described above, and for phytic acid P using the "ferric precipitation" method as described by Raboy et al. (1984). Briefly, samples were extracted in 0.4 *M* HCl: 10% (w/v) sodium sulfate. Following centrifugation, supernatant phytic acid was precipitated as a ferric salt. Ferric phytates were washed, wet ashed, and digest phytic acid phosphorus content determined colorimetrically using the method as described (Chen et al., 1956). Phytic acid P (186 g per mole of phytic acid) is converted to phytic acid (MW 660) by multiplying by the conversion factor 3.5484.

RESULTS AND DISCUSSION

Initial screening identified seed high in inorganic P (HIP), based on color intensity observations, from several M2 plants. Additional seeds from these plants and full-sibs of these plants were then screened for HIP. These screenings determined that seeds from M2 plant M153 were segregating for high versus low inorganic P (Table 1). Seeds produced on two seedling progeny from M2 plant M153 indicated that progeny M153-1 produced seeds both high and low in inorganic P. The M4 progenies of plant M153-1 also produced plants that were both high and low in inorganic P, suggesting that M153-1 was heterozygous for loci controlling this trait.

Progenies of the M4 plant M153-1-1 were all low in inorganic P in the M5 generation (Table 1). All four M5 progenies bred true for low inorganic P in the M6 generation. Means and standard errors for inorganic P for the four M4 plants varied from 0.29 ± 0.01 to 0.42 ± 0.07 g kg^{-1} P, based on spectrophotometer evaluations. The cultivar Athow averaged 0.21 ± 0.03 g kg^{-1} P.

Progenies of M4 plant M153-1-4 were all high in inorganic P (Table 1). Sixteen M5 progeny rows were grown, and 15 of these progeny rows produced plants all high in inorganic P. One progeny row, M153-1-4-6, segregated for plants both high and low in inorganic P. Quantities of inorganic P among all M6 plants varied from a low of 2.34 to a high of 4.69 g kg^{-1} P in the seed.

A second M2 plant, designated M766, produced progeny that were both high and low for inorganic P (Table 2). Progenies of M3 plants high in inorganic P were evaluated in the M4 through M6 generations. M4 plants M766-3-5, -3-8, -8-3, -8-4, and -8-5 produced lines all high in inorganic P. In the M5 generation, several of these lines bred true for high inorganic P and a few lines were segregating for this trait. Amounts of inorganic P among all HIP plants varied from 1.22 to 3.84 g kg^{-1} P in the seed, lower than the quantities observed among progenies of M153.

As a routine test to determine if these heritable increases in seed inorganic P are matched by equivalent reductions in seed phytic acid, individual seeds from selected progenies were extracted, and these extracts tested for both phytic acid P using HPLC, and inorganic P using the colorimetric assay. Typical results (Table 3)

Table 1. Distribution of plants producing seed with high or low inorganic P concentrations in succeeding generations of soybean mutant line M153, and for the cultivar Athow.

Generation	Plant designation	Progeny segregation	Range in M6 plant means for P
			g kg^{-1} seed
Athow			0.21†
M2	M153	–	
M3	M153-1	1 low: 1 high: 2 segregating	
M4	M153-1-1	All low	0.20†
M5	M153-1-1-2	13 low	0.21 to 0.38
	-3	6 low	0.26 to 0.38
	-5	4 low	0.24 to 0.79
	-9	10 low	0.28 to 0.37
M4	M153-1-4	All high	0.36†
M5	M153-1-4-2	14 high	2.94 to 3.98
	-3	9 high	2.82 to 3.90
	-6	22 low: 5 high: 3 segregating	
	-7	11 high	2.39 to 3.67
	-8	8 high	2.96 to 4.09
	-9	7 high	2.88 to 4.69
	-10	11 high	3.15 to 3.96
	-13	8 high	2.64 to 3.36
	-14	13 high	2.42 to 3.76
	-15	5 high	3.24 to 3.88
	-17	5 high	3.31 to 3.99
	-20	4 high	2.91 to 4.41
	-22	5 high	3.27 to 4.23
	-23	3 high	2.73 to 3.48
	-25	5 high	2.42 to 3.76
	-30	9 high	2.34 to 3.72

† Value for individual plant.

Table 2. Distribution of plants producing seed with high or low inorganic P concentrations in succeeding generations of soybean mutant line M766, and for the cultivar Athow.

Generation	Plant designation	Progeny segregation	Range in M6 plant means for P
			mg P kg^{-1} seed
Athow		low	0.19†
M2	M766	–	
M3	M766-3	1 low: 2 high	
M4	M766-3-4	All low	0.23†
	M766-3-5	All high	2.84†
M5	M766-3-5-1	16 high	1.96 to 3.34
	-4	4 low: 9 high: 2 segregating	
	-15	12 high	2.06 to 3.24
M4	M766-3-8	All high	2.18†
M5	M766-3-8-8	6 high	1.22 to 3.20
	-9	5 high	2.32 to 3.15
		26 low: 2 high: 4 segregating	
M3	M766-8	All high	2.74†
M4	M766-8-3	All high	2.95†
M5	M766-8-3-2	5 high: 1 low	
	-4	7 high	1.94 to 3.13
	-6	25 low: 4 high: 1 segregating	
	-18	4 high	2.70 to 2.95
	-19	8 high	2.34 to 3.35
	-21	7 high	2.30 to 3.24
	-22	5 high	2.61 to 3.06
	-23	10 high	2.09 to 3.84
	-24	6 high	0.62 to 0.84
M4	M766-8-4	All high	3.21†
M5	M766-8-4-8	16 low: 3 high: 1 segregating	
	-17	8 high	1.95 to 3.01
	-20	4 high	2.38 to 3.12
	-22	10 high	2.03 to 3.19
	-23	5 high	2.03 to 3.24
M4	M766-8-5	All high	
M5	M766-8-5-4	6 high	1.87 to 3.02
	-12	6 high	2.60 to 3.32
	-15	9 high	1.90 to 3.68
	-18	7 high: 1 segregating	
	-20	8 high	1.84 to 2.80
	-21	9 high	1.25 to 3.21
	-22	9 high	1.99 to 2.92
	-23	4 low: 9 high: 1 segregating	
	-24	2 low: 1 segregating	

† Value for individual plant.

Table 3. Correspondence between increased inorganic P and decreased phytic acid P, as determined by anion-exchange HPLC, in individual M5 soybean seeds of selected M4 parents.

M4 plant designation	Inorganic P phenotype	M5 single seed test	Phytic acid P	Inorganic P	Phytic acid P + Inorganic P
			g kg^{-1}		
M153-1-1	Low	1	4.43	0.18	4.61
		2	4.19	0.22	4.41
M153-1-4	High	1	0.82	3.14	3.96
		2	0.88	3.88	4.76
M766-3-8	Low	1	4.52	0.64	5.16
		2	4.95	0.28	5.23
M766-3-12	High	1	0.75	4.62	5.37
		2	1.49	3.52	5.01

of these analyses show two M4 progenies that appeared to be homozygous nonmutant (low seed inorganic P), M153-1-1 and M766-3-8, and two M4 progenies that appeared to be homozygous mutant (HIP), M153-1-4 and M766-3-12. The seed phytic acid P and inorganic P levels of M153-1-1 and M766-3-8 were similar to values expected of nonmutant soybean seeds (Table 3). In M153-1-4 seed, phytic acid P appears to be reduced 80%, as compared with its sibling nonmutant line M153-1-1, and this reduction in phytic acid P is essentially matched by an equivalent, in terms of P, increase in inorganic P. As a result, the sum of phytic acid P and inorganic P in M153-1-4 is similar to that of M153-1-1. Similar results were observed for M766-3-12 (mutant) when compared with M766-3-8 (nonmutant). In these and other results of the single-seed HPLC tests of these progenies, increases in seed inorganic P were in every case matched by equivalent decreases in phytic acid P, so that the sum of the two remains constant. This conclusion was supported by the fact that these HPLC analyses revealed no unusual accumulations of other *myo*-inositol polyphosphates related to phytic acid, such as *myo*-inositol pentaphosphates (data not shown).

These results were confirmed in an analysis of seeds produced by selected M6 plants both high and low for seed inorganic P (Table 4). Seeds of selected M6 plants, both low and high in inorganic P, were analyzed for phytic acid P using a second method, ferric precipitation. As observed before, the sum of seed phytic acid P and inorganic P remained relatively constant across the check cultivar Athow, and both mutant and nonmutant progenies of M153 and M766. The overall mean value was 5.42 g phytic acid P + inorganic P kg^{-1}, and the range was 4.73 to 6.23 g kg^{-1} (Table 4). Those progenies producing seed with high inorganic P therefore were low-phytate. The M6 progenies of M2 plant M153 that were high in inorganic P, were low in phytic acid and all had less phytic acid P than inorganic P. Inorganic P of these progenies varied from 60 to 70% of the sum of phytic acid P + inorganic P. The M6 progenies of M766 had about half of the seed P as inorganic P, somewhat less than that of the progenies of M153. Seeds of M766-3-5-4-156 were from a plant with low seed inorganic P. Both phytic acid and phytic acid P from this plant were very high compared to values for progenies of M153-1-4 or M766-8. In comparison, in seeds of Athow inorganic P represented only 15% of the sum of phytic acid P + inorganic P.

These results indicate that two independent, heritable and nonlethal soybean *low phytic acid* mutants have been isolated in progenies descending from M2 plants M153 and M766. These are phenotypically similar to the *low phytic acid* 1 mutants previously reported in corn, barley, and rice (Ertl et al., 1998; Larson et al., 1998, Larson et al., 2000; Raboy and Gerbasi, 1996). In such mutants, seed phytic acid P is reduced by 50% or more, as compared with wild-type or nonmutant seed, and this reduction is matched almost entirely by an equivalent increase in seed inorganic P, so that there is little or no effect on seed total P. When expressed as a percent of seed total P or percentage of the sum of phytic acid P and inorganic P, the reductions in soybean seed phytic acid P, and accompanying increases in inorganic P, are proportional in magnitude to those observed in cereal grain low phytic acid mutants. However, since soybean seed typically contain 50% or more seed total P than do cereal grains, in absolute terms the amount of P represented by these changes in seed P chemistry

Table 4. Seed phytic acid, phytic acid P, and inorganic P of Athow and of selected soybean mutants.

Mutant line	Phytic acid	Phytic acid P	Inorganic P	Phytic acid P + Inorganic P	Inorganic P
	g kg^{-1}				%
Athow	15.33	4.32	0.74	5.06	15
153-1-4-3-48	6.67	1.88	3.35	5.23	64
M153-1-4-8-69	6.28	1.77	2.96	4.73	63
M153-1-4-9-77	6.81	1.92	2.88	4.80	60
M153-1-4-15-94	6.81	1.92	3.74	5.66	66
M153-1-4-17-100	7.70	2.17	3.31	5.48	60
M154-1-4-25-114	6.74	1.90	3.76	5.66	66
M766-3-5-4-156	17.25	4.86	0.61	5.47	11
M766-8-3-2-219	11.99	3.38	2.85	6.23	46
M766-8-5-18-369	10.61	2.99	3.11	6.10	51
M766-8-6-4-419	10.08	2.84	2.42	5.26	46

are far greater than that observed in the cereal mutant. As an example, the increases in inorganic P concentrations in M153-1-4 or M766-3-12 represent an amount of P greater than the concentration of total P typically found in most cereal grains.

While the development of low phytic acid feed grains such as corn and barley may contribute to improved management of P in livestock production, the fullest value of this crop genetics and breeding approach to improved P management requires the development soybean lines with reduced phytic acid P, since most livestock rations consist of a cereal grain and soymeal. Animal trials evaluating low phytic acid grains have confirmed that the amount of seed total P that is "available P" for nonruminants basically consists of the total of seed nonphytic acid P, and "nonavailable P" is phytic acid P (Ertl et al., 1998; Huff et al., 1998; Pierce et al., 1998; Veum et al., 1998). It is therefore likely that in seed produced by these soybean low phytic acid mutants, "available P" is probably about 75% of seed total P, as compared with approximately 25% of seed total P that would be predicted for nonmutant soybean seed. In standard rations for poultry or swine, the soy meal component can contribute up to approximately 50% of the phytic acid P and total P in the feed, not counting supplementary P. Therefore, soybean lines developed using these mutants, or similar genetic resources, would represent an improved source of P for animal feeds. Use of such seed would reduce the need for P supplementation of feeds, or reduce the need for phytase supplementation. In addition, use of such seed in feeds would represent a valuable tool in reducing the amount of phytic acid P in animal waste, contributing to the current efforts to improve the management of P in agricultural production and efforts to reduce the impact of this production on the environment. Soybean low phytic acid lines may also have value in other uses of soybean, both in human foods and in industrial applications.

REFERENCES

Adeola, A. 1995. Digestive utilization of minerals by weanling pigs fed copper- and phytase-supplemented diets. Can. J. An. Sci. 75: 603–610.

Adeola, A., B.V. Lawrence, A.L. Sutton, and T.R. Cline. 1995. Phytase-induced changes in mineral utilization in zinc-supplemented diets for pigs. J. Anim. Sci. 73:3384–3391.

Bernard, R.L. 1966. Registration of Wayne soybeans. Crop Sci. 6:305.

Brown, K.H., and N.W. Solomons. 1991. Nutritional problems of developing countries. Infectious Disease Clinics of North America 5:297–317.

Chen, P.S., T.Y. Toribara, and H. Warner. 1956. Microdeterminations of phosphorous. Anal. Chem. 28:1756–1758.

Erdman, J.W., Jr. 1979. Oilseed phytates: nutritional implications. J. Am. Oil Chem. Soc. 56:736–741.

Erdman, J.W., Jr. 1981. Bioavailablity of trace minerals from cereals and legumes. Cereal Chem. 58:21–26.

Ertl, D.S., K.A. Young, and V. Raboy. 1998. Plant genetic approaches to P management in agricultural production. J. Environ. Qual. 27:299–304.

Huff, W.E., P.A. Moore, P.W. Waldroup, A.L. Waldroup, J.M. Balog, G.R. Huff, N.C. Rath, T.C. Daniel, and V. Raboy. 1998. Effect of dietary phytase and high available phosphorous corn on broiler chicken performance. Poultry Sci. 77:1899–1904.

Larson, S.R., K.A. Young, A. Cook, T.K. Blake, and V. Raboy. 1998. Linkage mapping of two mutations that reduce phytic acid content of barley grain. Theor. Appl. Genetics 97:141–146.

Larson, S.R., J.N. Rutger, K.A. Young, and V. Raboy. 2000. Isolation and genetic mapping of a non-lethal rice *low phytic acid 1* mutation. Crop Sci. 40:1397–1405.

Lei, X.G., P.K. Ku, E.R. Miller, M.T. Yokoyama, and D.E. Ullray. 1993. Supplementing corn-soybean meal diets with microbial phytase maximizes phytate P utilization of weanling pigs. J. Anim. Sci. 71:3368–3375.

Lonnerdal, B.L., Jayawickrama, and E. Lien. 1999. Effect of reducing the phytate content and of partially hydrolyzing the protein in soy formula on zinc and copper absorption and status in infant rhesus monkeys and rat pups. Am. J. Clin. Nutr. 69:490–496.

McCance, R.A., and E.M. Widdowson. 1935. Phytic acid in human nutrition. Biochem. J. 29:42694–42699.

Mendoza, C., F.E. Viteri, B.L. Lonnerdal, K.A. Young, V. Raboy, and K.H. Brown. 1998. Effect of genetically modified, low-phytic acid maize on absorption of iron from tortillas. Am. J. Clin. Nutr. 68:1123–1128.

Phillippy, B.Q., and J.M. Bland. 1988. Gradient ion chromatography of inositol phosphates. Anal. Biochem. 175:162–166.

Pierce, J.L., G.L. Cromwell, and V. Raboy. 1998. Nutritional value of low-phytic acid corn for finishing pigs. J. Anim. Sci. 76 (Suppl. 1):177.

Raboy, V. 1997. Accumulation and storage of phosphate and minerals. p. 441–477. *In* B.A. Larkins, and I.K. Vasilis (ed.) Cellular and molecular biology of plant seed development. Kluwer Academic Pub., Dordrecht, Netherlands.

Raboy, V., and D.B. Dickinson. 1987. The timing and rate of phytic acid accumulation in developing soybean seeds. Plant Physiol. 85:841–844.

Raboy, V., D.B. Dickinson, and F.E. Below. 1984. Variation in seed total P, phytic acid, zinc, calcium, magnesium, and protein among lines of *Glycine max* and *G. sojae*. Crop Sci. 24:431–434.

Raboy, V., and P. Gerbasi. 1996. Genetics of myo-inositol phosphate synthesis and accumulation. p. 257–285. *In* B.B. Biswas and S. Biswas (ed.) Myo-inositol phosphates, phosphoinositides, and signal transduction. Plenum Press, New York.

Rasmussen, S.K., and F. Hatzack. 1998. Identification of two low-phytate barley (*Hordeim vulgare* L.) grain mutants by TLC and genetic analysis. Hereditas 129:107–112.

Rounds, M.A., and Nielsen, S.S. 1993. Anion-exchange high-performance liquid chromatography with post-column detection for the analysis of phytic acid and other inositol phosphates. J. Chrom 653:148–152.

Sugiura, S.H., V. Raboy, K.A. Young, F.M. Dong, and R.W. Hardy. 1999. Availability of phosphorous and trace elements in low-phytate varieties of barley and corn for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 170:285–296.

Veum, T., V. Raboy, D. Ertl, and D. Ledoux. 1998. Low phytic acid corn improves calcium and phosphorous utilization for growing pigs. J. Anim. Sci. 76 (Suppl.)1:177.

Weber, C.R. 1966. Registration of Amsoy soybeans. Crop Sci. 6:611–612.

Wilcox, J.R., and T.S Abney. 1997. Registration of 'Athow' soybean. Crop Sci. 37:1981–1982.

Williams, J.H., J.E. Specht, A.F. Dreier, R.S. Moomaw, and L.V. Svec. 1980. Registration of Nebsoy soybeans. Crop Sci. 20:416.